

# Individualized Medicine

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## Individualized Medicine

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### Abstract

The recent focus of microelectromechanical-systems- (MEMS-) based instrumentation has largely dealt with increasing the throughput of established processes, including drug screening/drug discovery/combinatorial chemistry, or the miniaturization of accepted bench-top instruments. The miniaturization and automation of procedures that were previously performed manually are included in these activities.

We suggest that BioMEMS instrumentation will adopt an additional direction, that of providing information and capabilities to the physician that are not available, today.

### Introduction

Microelectromechanical-systems- (MEMS-) based instrumentation has enabled the reduction of size and cost of laboratory and clinical instrumentation, and, via the integration of functions, has increased the complexity of tasks that a single unit can perform. See, amongst others,

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[www.nanogen.com/tech.htm](http://www.nanogen.com/tech.htm)

[www.orchidbio.com](http://www.orchidbio.com)

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While these advances in the field of instrumentation are both encouraging and exciting, the revolutions in genomics and in the exploitation of databases on the Internet are inevitably pushing toward a revolution in medicine. The current mode of medicine might be referred to (disparagingly!) as “one size fits all”. The time is coming wherein medical practice will almost certainly evaluate and treat each person’s unique situation – “individualized medicine”.

There are likely to be at least two stages along the path to individualized medicine – “Stage I” will determine the genetic ID of an ailment, and “Stage II” will also determine the genetic information of the patient. The former will aid in determining the etiologic agents that are producing the patient’s symptoms, and the latter stage will provide key information that, one can reasonably expect, will broaden the number of available treatments for an affliction, as well as their efficacy. Each of these stages will be performed, within hours or less, by the technical personnel of the medical office or clinic.

### **Stage I**

One generalized, “near-term” goal for individualized medicine would be to determine the ailment of a patient while he/she is in the physician’s office

- Viral
- Bacterial (including antibiotic resistance or susceptibility, etc.)
- Protozoan, fungal, helminth, etc.
- None of the above (identification of allergies may require Stage II).

This requires the physician to have the ability to detect and determine the nucleic-acid sequences of the relevant organisms and to have a network over which the information may be exchanged in a timely fashion. The genetic identification of an ailment is likely to depend upon PCR, in one of its numerous embodiments.

## **PCR**

Although antibody-based assays and culture methods have been relied upon for decades, the polymerase chain reaction (PCR) has emerged(1-3) as a powerful tool in the arsenal of assays against both the established disease organisms, such as HIV(4), and emerging threats(5). Real-time PCR has added increased sensitivity, specificity, and rapidity of the assay (6-8). Because PCR (or reverse transcriptase, followed by PCR) reads the “book of life” for organisms from viral through human, we believe that it will play the key role in moving us towards individualized medicine. A few examples follow.

## **Influenza**

Researchers from the Center for Disease Control and Prevention, Influenza Branch, have said(9) that it would be valuable to have physicians be able to determine the genomic sequences of polymorphic regions for the strain(s) of influenza that infect our population. Each year, the CDC analyzes data on strains (10-14), including drug resistance(15), and they prepare a vaccine against such. As winter arrives, and as soon as cases start to appear, they would like to determine if there is a match between the vaccine that they have distributed and the flu strain(s) that are spreading through our population. The origin and spread of diseases, in general, is an important health issue in the US and other countries(16).

## **E. coli**

Taking a more general perspective than just influenza infections, such questions as “is an infection viral or bacterial or parasitic or a combination” need to be answered to determine the optimal treatment. Many bacterial and viral infections in humans manifest themselves with fever, often accompanied by cough and/or nausea. For the physician, the diagnostic difficulties presented by such similar symptoms may be considerable. For example, in a recent publication in the New England Journal of Medicine(17), Phillip Tarr and co-workers reported “Antibiotic treatment of children with *E. coli* O157:H7 infection increases the risk of the hemolytic-uremic syndrome” (HUS). Providing the

physician with a positive identification of the *E. coli* O157:H7 infection, and as quickly as possible, is clearly in order to avoid increasing the risk of HUS.

Peter Feng describes the difficulties in determining the presence of *E. coli* O157:H7 and its emerging phenotypic variants(18). Dr. Feng reports that such non-DNA-based assays as non-fermentation of sorbitol or methyl-umbelliferyl glucuronide assay have been “used extensively to distinguish the serotype O157:H7 from related bacteria.” However, he reports “Other enteric bacteria, such as *E. hermannii* and *Hafnia* spp., . . . resemble serotype O157:H7 on sorbitol-containing medium. Likewise, strains of O157, of non-H7 serotype that are not pathogenic and do not ferment sorbitol have occasionally been isolated . . .”. Dr. Feng then describes a more powerful assay for the presence of the *uidA* gene that is unique only to serotype O157:H7, based on PCR. He also states

Advantages of these new molecular methods include specificity, sensitivity, and the ability to detect phenotypic variants of the serotype O157:H7. However, these assays are far too complex and costly for use in the routine analysis of food or clinical specimens.

This last sentence should be taken as the “marching orders” for those of us whose goal is individualized medicine.

### **Assays Based on Combinations of Techniques**

One of the encouraging developments, in terms of both assays and instrumentation, has been the use of PCR with flow cytometry to increase the throughput of the analysis(19,20), including multiplexing(21).

### **Sample Preparation**

PCR assays, themselves, are clearly being relied upon to a greater extent. However, it is non-trivial to include automated sample collection and sample preparation, integrated with the PCR assay, in a portable instrument(22). One technique that performs sample cleanup or separations in microfabricated fluidic systems is dielectrophoresis(23). The process of dielectrophoresis uses an imposed electric field to induce an electric dipole in a molecule or cell, based on its polarizability. The imposed field is intentionally non-uniform, and those molecules or cells with the larger dipoles experience a force that draws them

towards the regions with higher electric fields. Since the voltages that can be applied to aqueous solutions are limited, even for alternating fields, by the onset of electrolysis of water, the reduced sizes of electrodes and flow channels in a microfabricated fluid system enhance the performance of dielectrophoresis(24-29). (The dielectrophoretic force is dependent upon the electric field and its gradient, not upon the voltage.)

## **Stage II**

A longer-term goal for individualized medicine would be the selection of treatment based on the genetics of each patient. This is a formidable technical challenge, but many of the required capabilities are being developed, already. It requires knowledge of the human genome and its polymorphisms. It requires a profound understanding of functional genomics - the relationship between an individual's genome and external stimuli, (the regulation of gene expression, the role of the proteins being synthesized, etc.). If attained, the results could be very beneficial. Armed with genetic information about infections and the patient, and after determining the biomarkers for allergies, exposure to toxins, as well as those for infections, the physician could select which medicines/treatments would be the most efficacious.

In order to achieve such a capability, science and technology both need to make major advances, but many researchers are moving in the necessary directions. The revolution in "information technology" also promises to provide the enormous increases in the bandwidth of information access and exchange that will be necessary to deliver such capabilities into the offices of physicians and into clinics. There will be a risk of "information overload", and there must be an expert system to assist the physicians in the use of such a complex databank/diagnostic function.

One existing reason that the cost of drug development is high is that a drug will be rejected in its final trials if a fraction of the cohort being studied shows sufficiently adverse reactions. The hope is that the genetic makeup of those who experience benefits from a drug may be delineated from those showing adverse reactions. This will permit the application of this drug to that fraction of the

population that will benefit. In the distant future, the science of functional genomics and proteomics may even permit the prediction of beneficial and adverse effects! (Stage III of individualized medicine?) Already, researchers are developing the high-throughput tools to examine polymorphisms in genes and their expression(30-32). Such techniques, that will probably include PCR, flow cytometry, and, possibly, mass spectroscopy, offer unprecedented power of analysis. One can project a time when an instrument will extract a small number of cells from a patient, possibly a blood sample, and analyze their genes and their response to stimuli in order to determine treatments to avoid and those to use – delivering individualized medicine to the patient.

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### **References**

1. Mullis K; Faloona F; Scharf S; Saiki R; Horn G; Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology*, 1986, **51**:263-73.
2. Mullis KB; Faloona FA. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods in Enzymology*, 1987, **155**:335-50.
3. Saiki RK; Gelfand DH; Stoffel S; Scharf SJ; Higuchi R; Horn GT; Mullis KB; Erlich HA. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 1988, **239**:487-91.
4. see, for example, Bennett JM; Kaye S; Berry N; Tedder RS. A quantitative PCR method for the assay of HIV-1 provirus load in peripheral blood mononuclear cells. *J. Virological Methods*, 1999, **83**:11-20.

5. The National Academy Press has recently released a volume entitled "Emerging Infections: Microbial Threats to Health in the United States, (J. Lederburg, R. E. Shope, and S. C. Oaks, Jr., Eds.).
6. Higuchi, R. Fockler, C., Dollinger, G., and Watson, R. (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. *Bio/Technology* **11**:1026-1030.
7. Belgrader P; Benett W; Hadley D; Richards J; Stratton P; Mariella R Jr; Milanovich F. PCR detection of bacteria in seven minutes. *Science*, 1999, **284**:449-50.
8. Belgrader P; Benett W; Hadley D; Long G; Mariella R Jr; Milanovich F; Nasarabadi S; Nelson W; Richards J; Stratton P. Rapid pathogen detection using a microchip PCR array instrument. *Clinical Chemistry*, 1998, **44**:2191-4.
9. Nancy J. Cox, CDC, Chief, Influenza Branch; Presentation at the National Academy of Sciences Colloquium on Infectious Disease, 29-30 April 1999. "Expanding the worldwide influenza surveillance system and improving the selection of strains for vaccines"
10. Brammer TL; Izurieta HS; Fukuda K; Schmeltz LM; Regnery HL; Hall HE; Cox NJ. Surveillance for influenza--United States, 1994-95, 1995-96, and 1996-97 seasons. *Morbidity and Mortality Weekly Report*. CDC Surveillance Summaries, 2000, **49**:13-28.
11. Bush RM; Smith CB; Cox NJ; Fitch WM. Effects of passage history and sampling bias on phylogenetic reconstruction of human influenza A evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 2000 Jun 20, **97**(13):6974-80.
12. Katz JM; Lim W; Bridges CB; Rowe T; Hu-Primmer J; Lu X; Abernathy RA; Clarke

- M; Conn L; Kwong H; et al.; Antibody response in individuals infected with avian influenza A (H5N1) viruses and detection of anti-H5 antibody among household and social contacts. *J. of Infect. Diseases*, 1999 , **180**:1763-70.
13. Bush RM; Bender CA; Subbarao K; Cox NJ; Fitch WM. Predicting the evolution of human influenza A. *Science*, 1999 , **286**:1921-5.
14. Xu X; Subbarao; Cox NJ; Guo Y. Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. *Virology*, 1999, **261**:15-9.
15. Ziegler T; Hemphill ML; Ziegler ML; Perez-Oronoz G; Klimov AI; Hampson AW; Regnery HL; Cox NJ. Low incidence of rimantadine resistance in field isolates of influenza A viruses. *J. of Infect. Diseases*, 1999, **180**:935-9.
16. James Hughes, CDC, Director, National Center for Infectious Diseases, spoke on emerging infectious diseases, including *Shigella sonnei* – outbreak in US from parsley grown in Mexico “. . . physicians need office-based tests - highest priority.”. Same colloquium as ref. 10.
17. C. S. Wond, S. Jelacic, R. L. Habeeb, S. L. Watkins, P.I. Tarr, *N.E. J. of Medicine*, 2000, **342**:1930-6.
18. Feng P. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerging Infectious Diseases*, 1995, **1**:47-52.
19. Dorenbaum A; Venkateswaran KS; Yang G; Comeau AM; Wara D; Vyas GN. Transmission of HIV-1 in infants born to seropositive mothers: PCR-amplified proviral DNA detected by flow cytometric analysis of immunoreactive beads. *J. Acquired Immune Deficiency Syndromes and Human Retrovirology*, 1997, **15**:35-42.

20. Gibellini DE; Re MC; Furlini G; La Placa M. Flow cytometry analysis of an in situ PCR for the detection of human immunodeficiency virus type-1 (HIV-1) proviral DNA. *Methods in Molecular Biology*, 1997, **71**:113-22.
21. M. A. Iannone, et al., "Multiplexed Single Nucleotide Polymorphism Genotyping by Oligonucleotide Ligation and flow Cytometry", *Cytometry*, 2000, **39**:131-140.
22. Belgrader P; Hansford D; Kovacs GT; Venkateswaran K; Mariella R Jr; Milanovich F; Nasarabadi S; Okuzumi M; Pourahmadi F; Northrup MA. A minisonicator to rapidly disrupt bacterial spores for DNA analysis. *Analytical Chemistry*, 1999, **71**:4232-6.
23. Pohl HA; *Dielectrophoresis*, Cambridge Univ. Press, Cambridge, 1978.
24. Talarly MS; Mills KI; Hoy T; Burnett AK; Pethig R. Dielectrophoretic separation and enrichment of CD34+ cell subpopulation from bone marrow and peripheral blood stem cells. *Medical and Biological Engineering and Computing*, 1995, **33**:235-7.
25. Markx GH; Dyda PA; Pethig R. Dielectrophoretic separation of bacteria using a conductivity gradient. *J. Biotechnology*, 1996, **51**:175-80.
26. Huang Y; Yang J; Wang XB; Becker FF; Gascoyne PR. The removal of human breast cancer cells from hematopoietic CD34+ stem cells by dielectrophoretic field-flow-fractionation [see comments]. *J. Hemat. Stem Cell Res*, 1999, **8**:481-90.
27. Schnelle T; Muller T; Hagedorn R; Voigt A; Fuhr G. Single micro-electrode dielectrophoretic tweezers for manipulation of suspended cells and particles. *Biochimica et Biophysica Acta*, 1999, **1428**:99-105.
28. Morgan H; Hughes MP; Green NG. Separation of submicron bioparticles by dielectrophoresis. *Biophysical Journal*, 1999, **77**:516-25.

29. Schnelle T; Muller T; Gradl G; Shirley SG; Fuhr G. Dielectrophoretic manipulation of suspended submicron particles. *Electrophoresis*, 2000, **21**:66-73.
30. Wieckiewicz J; Krzeszowiak A; Ruggiero I; Pituch-Noworolska A; Zembala M. Detection of cytokine gene expression in human monocytes and lymphocytes by fluorescent in situ hybridization in cell suspension and flow cytometry. *International J of Molecular Medicine*, 1998 , **1**:995-9.
31. Jacobberger JW; Sramkoski RM; Wormsley SB; Bolton WE. Estimation of kinetic cell-cycle-related gene expression in G1 and G2 phases from immunofluorescence flow cytometry data. *Cytometry*, 1999, **35**:284-9.
32. Gaynor EM; Mirsky ML; Lewin HA. Use of flow cytometry and RT-PCR for detecting gene expression by single cells. *Biotechniques*, 1996, **21**:286-91.